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## Human T-Cell Lymphotropic Virus Type III: Immunologic Characterization and Primary Structure Analysis of the Major Internal Protein, p24

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The major internal structural protein of human T-cell lymphotropic virus type III (HTLV-III), a virus etiologically implicated in acquired immunodeficiency syndrome (AIDS), was purified to homogeneity. This 24,000-molecular-weight protein (p24) was shown to lack immunologic cross-reacting antigenic determinants shared by other known retroviruses, including HTLV-I and HTLV-II, with the exception of equine infectious anemia virus (EIAV). A broadly reactive competition immunoassay was developed in which antiserum to EIAV was used to precipitate <sup>125</sup>I-labeled HTLV-III p24. Although the major structural proteins of HTLV-III and EIAV competed in this assay, other type B, C, and D retroviral proteins lacked detectable reactivity. Thus, HTLV-III is more related to EIAV than to any other retroviruses. That the HTLV-III isolate is very distinct from HTLV-I and HTLV-II was further confirmed by the amino acid compositions of the major internal antigens of all three isolates. Moreover, comparison of the amino-terminal amino acid sequence of HTLV-III p24 with analogous sequences for HTLV-I and HTLV-II p24 showed that these proteins do not share significant sequence homology. In an attempt to evaluate immune response in individuals exposed to HTLV-III, sera from AIDS and lymphadenopathy syndrome patients as well as from clinically normal blood donor controls were tested for antibodies to HTLV-III p24. The results showed that sera from 93% of lymphadenopathy syndrome patients and 73% of AIDS patients exhibited high-titered antibodies to HTLV-III p24. In contrast, none of the normal control sera showed detectable reactivity to HTLV-III p24.

Human retroviruses have been implicated as etiologic agents in acquired immunodeficiency syndrome (AIDS). To date, there are several independent reports of retrovirus isolations from patients with AIDS or lymphadenopathy syndrome (LAS). These viruses include lymphadenopathy-associated virus (LAV; 7), several isolates designated as human T-cell lymphotropic viruses (HTLV) type III (HTLV-III; 19, 39), AIDS-associated retrovirus (ARV-2; 30), and three isolates derived from AIDS patients (17). Serologic and molecular hybridization studies have indicated that all these viruses are closely related (12, 31; unpublished data), though they may show diversity in the genomic restriction site maps (1, 46; S. G. Devare, manuscript in preparation). The frequent isolation of retroviruses from AIDS patients, seroepidemiologic studies, and infectivity studies on chimpanzees with LAV resulting in a characteristic decrease in T-cell ratio (18) indicate that the LAV-HTLV-III group of viruses is the etiologic agent implicated in AIDS (17, 19, 30).

Analysis of LAV-HTLV-III structural proteins has led to the demonstration of three glycosylated proteins with molecular weights around 160,000, 120,000, and 41,000, whereas the nonglycosylated proteins include proteins with molecular weights around 55,000, 24,000, 18,000, and 15,000 (28, 42; Devare et al., in preparation). Major internal proteins of the retroviruses have been useful in developing specific and sensitive immunoassays which have diagnostic application. The presence of antibodies to retroviral antigens in the sera of animals exposed to viruses has been reported in several model systems (10, 15, 16, 27). Apart from their utility in specific immunoassays, biochemical and immunologic characterizations of retroviral major internal pro-

teins have been useful in correlating various isolates from diverse species of animals and the evolutionary relationships of these isolates to each other (2, 5, 6, 11, 13, 37, 44, 47).

The present studies were undertaken in an effort to biochemically and immunologically characterize the major internal protein of HTLV-III. In addition, the relationships between other members of the HTLV group of viruses, including HTLV-I and HTLV-II, were assessed by immunologic as well as amino-terminal amino acid sequence analyses. Furthermore, the utility of <sup>125</sup>I-labeled HTLV-III major internal protein in the detection of an immune response to HTLV-III in individuals with AIDS or LAS and in normal blood donors was evaluated.

### MATERIALS AND METHODS

**Virus.** Human T-cells chronically infected with HTLV-III (HT-9, established by R. C. Gallo) were provided by L. Arthur, Frederick Cancer Research Facility, Frederick, Md. The virus was concentrated by density gradient centrifugation from cell culture fluids of the HT-9 cells. The sources of other retroviruses utilized, including Rauscher murine leukemia virus, *Papio cynocephalus* baboon virus, simian sarcoma-associated virus, Mason-Pfizer monkey virus, squirrel monkey virus, bovine leukemia virus (BLV), and mouse mammary tumor virus, have been previously described (13). Purified equine infectious anemia virus (EIAV), HTLV-I, and HTLV-II were provided by V. S. Kalyanaraman, Centers for Disease Control, Atlanta, Ga.

Sera. Antisera to detergent-disrupted HTLV-III were prepared in goats and rabbits by immunizing the animals with 1 mg of detergent-disrupted, ether-extracted virus per ml as described previously (13). Antisera to HTLV-I and HTLV-II were prepared in rabbits by a similar procedure. Sources of

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antisera to other retroviruses have also been described previously (13). Horse serum with high-titered antibodies to EIAV structural proteins was kindly provided by J. Miller, U.S. Department of Agriculture, Ames, Iowa. Human sera from AIDS and LAS patients were obtained from G. Gitnick, University of California at Los Angeles, Los Angeles, and sera from normal individuals were obtained from the Illinois Blood Bank, Chicago.

**Isolation of HTLV-III major structural protein.** The major HTLV-III structural protein was isolated by phosphocellulose column chromatography as described previously (13, 15, 26). About 10 mg of virus was disrupted by sonication in 0.05 M Tris-hydrochloride (pH 9.0) buffer containing 1% Triton X-100. After 15 min of incubation at room temperature, samples were clarified by centrifugation at 100,000  $\times$  g for 30 min, dialyzed overnight against 0.01 M *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (pH 6.5)–1.0 mM EDTA–0.1% Triton X-100 (BET buffer), and applied to a Celex column (1.6 by 5.0 cm; Bio-Rad Laboratories, Richmond, Calif.) equilibrated with BET buffer. The column was washed with 50 ml of a gradient of 0 to 1.0 M NaCl in BET buffer at 4°C. Fractions containing a single protein band with a molecular weight of about 24,000, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (29), were pooled and stored under liquid nitrogen. This protein was used for the development of radioimmunoassays. For amino acid composition and amino-terminal amino acid sequence determination, the protein was further purified by high-pressure liquid chromatography (14, 36).

**Radioimmunoassay.** The 24,000-molecular-weight major internal structural protein of HTLV-III was labeled with  $^{125}$ I at high specific activity ( $\sim 30$   $\mu$ Ci/ $\mu$ g) by the IODO-BEADS (Pierce Chemical Co., Rockford, Ill.) method (32). Immunoprecipitation of  $^{125}$ I-labeled protein was performed by the double-antibody technique (15, 44, 47). Antisera were assayed at serial twofold dilutions for their ability to precipitate approximately 10,000 cpm of  $^{125}$ I-labeled antigen in 0.2 ml of reaction mixture containing 0.01 M Tris-hydrochloride (pH 7.8), 1.0 mM EDTA, 0.01 M NaCl, 1% bovine serum albumin, and 0.4% Triton X-100. Reaction mixtures were incubated for 3 h at 37°C and for a further 18 h at 4°C. A 0.025-ml volume of appropriate anti-immunoglobulin G was added to each tube to precipitate the antigen-antibody complexes, and the samples were further incubated for 1 h at 37°C and for 3 h at 4°C. After the addition of 0.4 ml of cold 10 mM Tris-hydrochloride (pH 7.8)–10 mM NaCl buffer containing 0.1% Triton X-100 to each tube, samples were centrifuged for 15 min at 2,500 rpm, supernatants were aspirated, and the radioactivity in the precipitates was determined in the ANSR gamma counter of Abbott Laboratories.

**Competition Immunoassay.** Competition immunoassays were performed by testing unlabeled viral antigens at serial twofold dilutions for their ability to compete with  $^{125}$ I-labeled viral structural proteins in binding limiting amounts of antiserum (5, 6, 13, 15). Antiserum and unlabeled competing antigen were incubated for 1 h at 37°C in reaction mixtures as described above, and then 10,000 cpm of  $^{125}$ I-labeled antigen was added. After further incubation for 3 h at 37°C and for 18 h at 4°C, antigen-antibody complexes were precipitated by the addition of 0.025 ml of the appropriate anti-immunoglobulin G.

**Amino acid composition analysis.** The HTLV-III major internal protein, purified by high-pressure liquid chromatography, was hydrolyzed in vacuo at 115°C for 24 h with 6 M HCl–0.03% phenol–0.01%  $\beta$ -mercaptoethanol. Hydrolyzed

protein was subjected to analysis on a Beckman 6300 amino acid analyzer. With the exception of cysteine and tryptophan, the amino acid composition was determined with a Spectra Physics 4100 integrator.

**Amino-terminal amino acid sequence analysis.** Amino acid sequence analysis was performed by automated Edman degradation on a gas-phase microsequencer model 470 from Applied Biosystems, Inc., Foster City, Calif. (22). The phenylthiohydantoin amino acids obtained from the automated sequencer were analyzed with a Beckman Altex 345 high-pressure liquid chromatography system with an Ultrasphere octadecylsilane reversed-phase column by the modified method of Black and Coon (8; J. Meuth, unpublished data).

## RESULTS

**Immunologic characterization of the HTLV-III major internal protein.** The major nonglycosylated HTLV-III internal protein was purified as described above. The purity of the HTLV-III major internal protein was demonstrated by radiolabeling the protein with  $^{125}$ I and analyzing it by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1). The HTLV-III major internal protein under reducing as well as nonreducing conditions migrated as a single band with a molecular weight of around 24,000 relative to protein standards. According to convention, this protein was designated HTLV-III p24 (4).

For immunologic characterization, hyperimmune sera against detergent-disrupted HTLV-III produced in goat and rabbit, as well as other test sera raised against various retrovirus isolates, including HTLV-I and HTLV-II, were

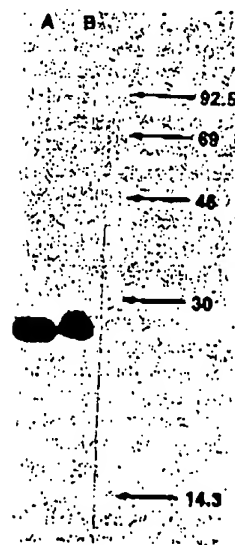


FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of  $^{125}$ I-labeled HTLV-III p24. Samples of about 100,000 cpm were subjected to electrophoresis on a 12% sodium dodecyl sulfate-polyacrylamide gel in the (A) presence or (B) absence of 2%  $\beta$ -mercaptoethanol.  $^{14}$ C-labeled molecular weight markers, including myosin (200,000), phosphorylase (92,500), bovine serum albumin (69,000), actin (46,000), carbonic anhydrase (30,000), and lysozyme (14,300), were coelectrophoresed for molecular weight determination. The gels were dried and autoradiographed with XAR-5 film for 4 h.

tested for immunoprecipitation of  $^{125}$ I-labeled HTLV-III p24. Also included in the studies was horse serum with high-titered antibodies to EIAV. Antiserum to HTLV-III bound  $^{125}$ I-labeled HTLV-III p24 at high titers ( $>1:200,000$ ) and to a final extent of over 95% at the lowest dilution (Table 1). In contrast, with the exception of horse antiserum to EIAV, which bound  $^{125}$ I-labeled HTLV-III p24 at a dilution of 1:100, high-titered antisera produced against several other retroviruses including HTLV-I and HTLV-II failed to bind HTLV-III p24 to a significant extent.

To determine the immunologic cross-reacting determinants between HTLV-III p24 and EIAV, a competition immunoassay with limiting concentrations of anti-HTLV-III for the precipitation of  $^{125}$ I-labeled HTLV-III p24 was developed. In this homologous competition immunoassay, detergent-disrupted HTLV-III competed efficiently at a high dilution (Fig. 2). Other retroviruses tested, including HTLV-I, HTLV-II, EIAV, Rauscher murine leukemia virus, *P. cynocephalus* baboon virus, BLV, mouse mammary tumor virus, squirrel monkey virus, and Mason-Pfizer monkey virus, lacked detectable reactivity. These results demonstrate that the HTLV-III major structural protein could be discriminated from EIAV by a homologous competition immunoassay for HTLV-III p24.

In an effort to characterize broadly reactive immunologic cross-reacting determinants between HTLV-III and other retroviruses, an immunoassay was developed in which the limiting concentration of anti-EIAV for the precipitation of  $^{125}$ I-labeled HTLV-III p24 was used. When various detergent-disrupted viruses were tested in this heterologous competition immunoassay, both HTLV-III and EIAV competed efficiently and at relatively high titers (Fig. 3). In contrast, none of the other viruses tested, including HTLV-I, HTLV-II, and various type B, C, and D retroviruses, showed detectable reactivity. These findings demonstrate that HTLV-III and EIAV share unique immunologic cross-reacting antigenic determinants.

**Amino acid composition and primary structure analyses of HTLV-III p24.** In an effort to further characterize HTLV-III p24, the protein was hydrolyzed in vacuo as described above. The hydrolyzed HTLV-III p24 was subjected to analysis on a Beckman 6300 amino acid analyzer. The previously published data on amino acid composition of

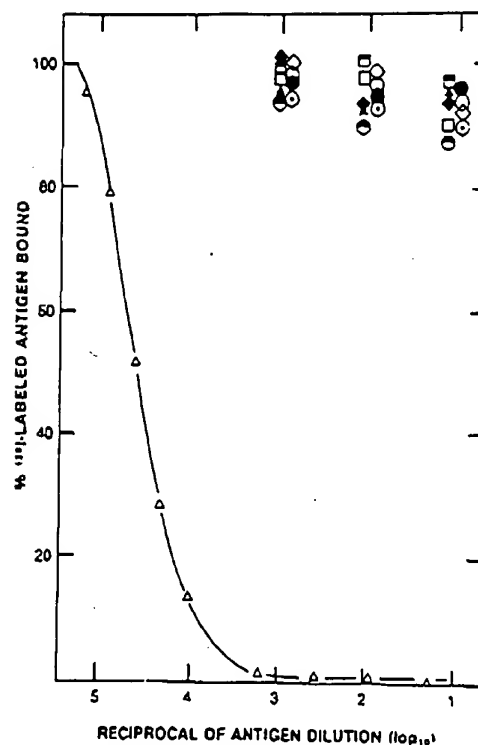


FIG. 2. Homologous competition immunoassay for HTLV-III p24. Detergent-disrupted viral antigens were tested at serial twofold dilutions for their ability to compete with  $^{125}$ I-labeled HTLV-III p24 in the binding of limiting amounts of goat anti-HTLV-III (1:20,000) as described in the text. Viruses tested included HTLV-III ( $\Delta$ ), HTLV-I ( $\triangle$ ), HTLV-II ( $\diamond$ ), Rauscher murine leukemia virus ( $\circ$ ), *P. cynocephalus* baboon virus ( $\square$ ), BLV ( $\blacksquare$ ), mouse mammary tumor virus ( $\circ$ ), Mason-Pfizer monkey virus ( $\bullet$ ), squirrel monkey virus ( $\circ$ ), and EIAV ( $\circ$ ).

TABLE 1. Comparison of abilities of sera to bind  $^{125}$ I-labeled HTLV-III p24

Antisera to <sup>a</sup> :	Titer for binding <sup>b</sup>	% Maximum bound <sup>c</sup>
HTLV-III	200,000	98
HTLV-I	<10	<5
HTLV-II	<10	<5
SMRV	<10	<5
MPMV	<10	<5
SSV-1-SSAV	<10	<5
M7	<10	<5
RD114	<10	<5
GalV	<10	<5
MMTV	<10	<5
BLV	<10	<5
EIAV	100	30

<sup>a</sup> Antisera to detergent-disrupted retroviruses were prepared in goats as described previously (13). SMRV, Squirrel monkey virus; MPMV, Mason-Pfizer monkey virus; SSV-1-SSAV, simian sarcoma-associated virus; M7, *P. cynocephalus* baboon virus; MMTV, mouse mammary tumor virus; GalV, gibbon ape leukemia virus.

<sup>b</sup> Serum titers are expressed as the reciprocals of the highest dilution capable of binding 10% of the  $^{125}$ I-labeled HTLV-III p24.

<sup>c</sup> At a serum dilution of 1:10.

HTLV-I p24 based on nucleic acid sequence analysis (45) and of HTLV-II p24 determined by an analysis of purified protein (14) were used for comparison with the HTLV-III p24 amino acid composition (Table 2). Although the amino acid compositions of HTLV-I p24 and HTLV-II p24 showed close similarities, the amino acid composition of HTLV-III p24 was substantially different from that of other HTLV groups of viruses. Thus, the lack of immunologic cross-reactivity between HTLV-III p24 and analogous proteins from HTLV-I and HTLV-II evident from immunologic analysis could be corroborated by the amino acid composition of these major internal proteins.

The conclusions based on amino acid composition can be very limited. Therefore, it was decided to determine the amino-terminal amino acid sequence of HTLV-III p24. The amino-terminal amino acid sequence of the first 30 residues of HTLV-III p24 is shown in Fig. 4. All the amino acids could be identified unambiguously by the high-pressure liquid chromatography analysis of phenylthiohydantoin derivatives released at each cycle in a microsequencer. The amino-terminal amino acid for HTLV-III p24 is proline, which is a conserved amino acid for several mammalian retroviral major internal proteins (35). In an effort to compare HTLV-III p24 with analogous proteins of other retroviruses, amino-terminal amino acid sequences of HTLV-I (45), HTLV-II (14), BLV, and feline leukemia virus (34) are

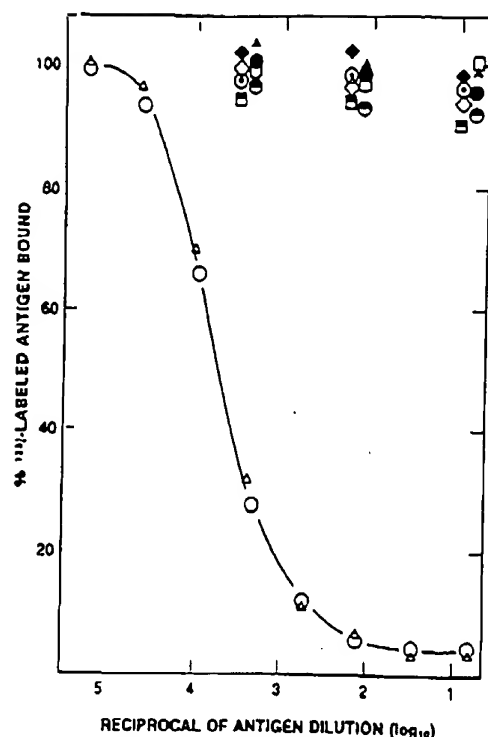


FIG. 3. Heterologous competition immunoassay for HTLV-III p24. Detergent-disrupted viral antigens were tested at serial twofold dilution for their ability to compete with  $^{125}\text{I}$ -labeled HTLV-III p24 in the binding of limiting amounts of horse anti-EIAV (1:30) as described in the text. Symbols of the viruses tested as competing antigens are listed in the legend to Fig. 2.

also shown in Fig. 4. A comparison of the amino acid sequence of HTLV-III p24 with the sequences of HTLV-I and HTLV-II major internal proteins showed five and six amino acid identities, respectively, in 30 amino-terminal amino acids. BLV, on the other hand, showed nine residues identical to those in the amino-terminal sequence of HTLV-III p24. Feline p30 showed only two residues identical to those of HTLV-III p24 in the amino-terminal sequence matched. These data indicate that, unlike HTLV-I and HTLV-II, which share a high degree of sequence homology in their amino-terminal sequences, HTLV-III p24 is significantly different from the major internal proteins of other known mammalian retroviruses.

**Antibodies to HTLV-III p24 in human sera.** Immunoprecipitation assays with  $^{125}\text{I}$ -labeled viral proteins are useful for detection of antibody in the sera of retrovirus-exposed animals. The immunoassay for HTLV-III p24 was applied to the detection of serologic reactivity against HTLV-III in various human sera. These sera were from groups of patients with AIDS and LAS and from clinically normal laboratory workers and blood donors. Antibodies to HTLV-III p24 were present in 73% of AIDS and 93% of LAS patients (Table 3). In contrast, laboratory workers and normal blood donors lacked detectable antibodies to HTLV-III p24.

#### DISCUSSION

To date, several retrovirus isolates designated as LAV, HTLV-III, and ARV-2 have been described as the etiologic agents in AIDS (7, 17, 19, 30). Accumulating evidence based

TABLE 2. Amino acid composition of the major internal proteins of HTLV-I, HTLV-II, and HTLV-III

Amino acid	No. of residues in:		
	HTLV-I <sup>a</sup>	HTLV-II <sup>b</sup>	HTLV-III <sup>c</sup>
Asp and Asn	19	17	17
Thr	9	12	15
Ser	13	12	12
Glu and Gln	30	30	28
Pro	18	23	18
Gly	11	14	18
Ala	20	18	19
Cys	3		
Val	9	8	12
Met	4	4	7
Ile	8	7	13
Leu	28	27	14
Tyr	5	5	5
Phe	4	5	5
His	10	10	9
Lys	8	5	5
Trp	4		
Arg	11	10	9

<sup>a</sup> Taken from Seiki et al. (45).

<sup>b</sup> Taken from Devare et al. (14).

<sup>c</sup> Calculations based on 206 amino acid residues in HTLV-III p24, with exclusion of Cys and Trp residues.

on serologic and genomic nucleic acid hybridization studies indicates that all these retroviruses are closely related (12, 31, 46; unpublished data). In the present study we have isolated the major 24,000-molecular-weight structural protein, p24, from HTLV-III. An effort was made to immunologically and biochemically characterize this protein and also to evaluate its utility in the detection of antibodies in sera from individuals exposed to HTLV-III.

The  $^{125}\text{I}$ -labeled HTLV-III p24 could be efficiently precipitated by the antiserum directed against HTLV-III. Although none of the other sera raised against various retroviruses, including type B, C, and D prototypes, could immunoprecipitate HTLV-III p24, antibodies to EIAV (a retrovirus

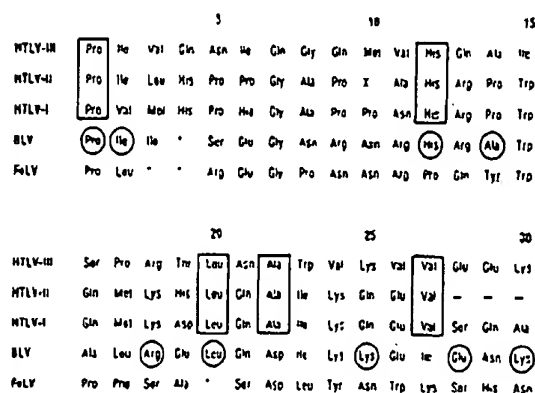


FIG. 4. Amino-terminal amino acid sequence comparison between HTLV-III, HTLV-I (45), HTLV-II (14), BLV, and feline leukemia virus (34) major internal proteins. The amino acid residues common in HTLV-I, HTLV-II, and HTLV-III are boxed, whereas those common between HTLV-III and BLV are highlighted by circles around the amino acid in the BLV p24 amino acid sequence. —, Gap introduced to maximize sequence homology; X, amino acid which could not be identified conclusively; —, amino acid not determined during sequence analysis.

associated with infectious anemia in the horse) precipitated  $^{125}\text{I}$ -labeled HTLV-III p24, though at a relatively low titer. The detergent-disrupted EIAV failed to show reactivity in a homologous competition immunoassay in which limiting concentrations of antibodies to HTLV-III were used to precipitate HTLV-III p24. However, in a heterologous competition immunoassay, in which limiting concentrations of EIAV antibodies were used to precipitate HTLV-III p24, only HTLV-III and EIAV could compete efficiently, while a broad range of other retroviruses failed to show detectable reactivity. These results demonstrate that HTLV-III is more closely related to EIAV than to any other known retrovirus. It is interesting to note that previous studies of EIAV have shown that it does not share antigenic determinants with other known retroviruses (9) or lentiviruses (48) and thus may have unique antigenic determinants. Furthermore, EIAV does not exhibit detectable nucleic acid sequence homology with other retroviruses (40). Similar to HTLV-III, EIAV is not endogenous to horses (40). These data confirm and extend the results of previous studies by Montagnier et al. (33), who have shown that the LAV internal protein (p25) can be immunoprecipitated by antibodies to EIAV and that LAV mature virion particles are morphologically similar to EIAV and type D viruses. More recently, on the basis of heteroduplex analysis and the morphology of budding particles during replication, HTLV-III has been shown to be related to visna virus, a retrovirus of sheep and a member of the lentivirus family (20). The immunologic cross-reactivity between HTLV-III and EIAV in the present studies suggests an evolutionary relationship between these viruses and provides some clues for the origin of HTLV-III. Further research may elucidate the potential relationship between these viruses.

HTLV-III, HTLV-I, and HTLV-II, have been classified as HTLV because of the preference of these groups of viruses to replicate in T cells. However, HTLV-III differs considerably from HTLV-I and HTLV-II. HTLV-III is typically a cytopathic virus that affects the cell-mediated immunity of the patient, resulting in a reduced subpopulation of helper T lymphocytes (7, 19). In contrast, HTLV-I and HTLV-II viruses are implicated in T-cell proliferation. The disease associated with HTLV-I is malignant adult T-cell leukemia (23, 38), whereas the disease associated with HTLV-II is a relatively benign form of leukemia (25). It was of interest, therefore, to compare the major internal proteins of HTLV-I, HTLV-II, and HTLV-III. The  $^{125}\text{I}$ -labeled HTLV-III p24, which could be efficiently recognized by antibodies to HTLV-III, failed to be bound by high-titered antibodies to HTLV-I and HTLV-II. Moreover, HTLV-I- and HTLV-II-associated antigens lacked detectable reactivity in either the

homologous competition immunoassay in which antibodies to HTLV-III were used to precipitate HTLV-III p24 or the heterologous competition immunoassay in which antibodies to EIAV were used to precipitate HTLV-III p24. Furthermore, the amino-terminal amino acid sequence analysis lacked significant sequence homology between the sequences of the major internal proteins of HTLV-III and HTLV-I or HTLV-II. Thus, HTLV-III p24 could be readily discriminated from the p24 of HTLV-I and HTLV-II on the basis of both antigenic and biochemical properties.

This lack of immunologic reactivity between HTLV-III and HTLV-I or HTLV-II major internal proteins agrees well with the previously reported observations on p25 from LAV. LAV p25 does not share immunologic cross-reacting determinants with HTLV-I, HTLV-II, or any other retroviruses (7, 24). However, previously reported data on the immunologic characterization of HTLV-III proteins have indicated that they are related to HTLV-II proteins (42, 43). These data were further supported by nucleic acid hybridization studies with cDNA probe derived from reverse transcription of HTLV-III genomic RNA which indicate that HTLV-III is more closely related to HTLV-II than to HTLV-I (3). The lack of binding of  $^{125}\text{I}$ -labeled HTLV-III p24 by antisera to HTLV-I and HTLV-II in the present studies could be due to titers of antibodies lower than those reported by Schüpbach et al. (43). However, our anti-HTLV-I and anti-HTLV-II sera showed high-titered antibodies to homologous proteins HTLV-I p24 and HTLV-II p24, respectively (data not shown). Moreover, previous reports on antigenic cross-reactivity between HTLV-I and HTLV-II major internal proteins could be readily demonstrated with these reagents (data not shown). More recently, the LAV genome has been molecularly cloned, and, with its nick-translated probe, Alizon et al. (1) failed to detect nucleic acid hybridization with HTLV-I or HTLV-II molecularly cloned DNAs. We are unable to resolve these conflicting data. However, the availability of molecularly cloned HTLV-III (21, 46), LAV (1), and ARV-2 (31) clones would be useful for the nucleotide sequence analyses which should indicate any possible sequence homology between various HTLV.

Major internal proteins from viruses have been used previously in various model systems to evaluate infection by retroviruses (10, 15, 16, 27). It was of interest, therefore, to assess whether it would be possible to detect immune response in human sera as a measure of exposure to HTLV-III. We could detect high-titered antibodies in sera from 73% of AIDS and 93% of LAS patients. In contrast, none of the clinically normal laboratory workers or blood donors showed detectable antibodies to HTLV-III p24.

There have been several reports on various methods for the detection of antibodies to HTLV-III-LAV isolates in human sera. Using disrupted HTLV-III-coated microtiter plates in an enzyme-linked immunoassay, Samgadharan et al. (42) demonstrated antibodies to HTLV-III in 87.8% of AIDS and 78.6% of pre-AIDS patients. In other studies, Western blotting analysis could detect antibodies to HTLV-III in 100% of AIDS and 84% of LAS patients (41). The radioimmunoassay for LAV major internal protein, p25, detected positive reactivity in 41% of AIDS and 72% of LAS patients (24). The radioimmunoassay in the present studies could detect antibodies to HTLV-III p24 in 73% of AIDS and 93% of LAS patients. These results may indicate that an HTLV-III p24 assay would provide a better means for the detection of antibodies in LAS patients, compared with other reported procedures. However, the differences in the

TABLE 3. Detection of antibody to  $^{125}\text{I}$ -labeled HTLV-III p24 in human sera

Status of patient	No. of human sera		Antiserum titer* range	% Positive
	Tested	With antibodies*		
AIDS	41	30	20->100,000	73
LAS	43	40	20->100,000	93
Normal	120	0	<10	0

\* To  $^{125}\text{I}$ -labeled HTLV-III p24.

\* Serum titers are expressed as the reciprocal of the highest dilution capable of binding 10% of  $^{125}\text{I}$ -labeled HTLV-III p24 and represent the mean of two separate determinations. Background precipitation of  $^{125}\text{I}$ -labeled HTLV-III p24 in the absence of antibody was never greater than 4%.

results of various assay procedures may be due to differences in the test panels, stage of the disease when sera were obtained, or quality of the antigen used. The detection of antibodies to HTLV-III in a lower percentage of AIDS patients in the present studies may be due to immunosuppression in AIDS patients who might have lost the ability to synthesize antibodies to HTLV-III proteins. Yet another explanation may be that these differences are due to heterogeneity in antigenic determinants from various isolates, some being antigenically superior to others. Recent evidence demonstrates significant restriction site polymorphism between various isolates (1, 46). Whether this polymorphism would result in antigenic variation in viral encoded proteins needs to be resolved by further experimentation.

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#### ADDENDUM IN PROOF

After submission of this manuscript, complete nucleotide sequences of molecular clones of retrovirus isolates LAV, HTLV-III, and ARV-2 were reported (S. Wain-Hobson, P. Sonigo, O. Danos, S. Cole, and M. Alizon, *Cell* 40:9-17, 1985; L. Ratner, W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Raffaski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, Jr., M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal, *Nature (London)* 313:277-284, 1985; and R. Sanchez-Pescador, M. D. Power, P. J. Barr, K. S. Steimer, M. M. Stempien, J. A. Brown-Shimer, W. W. Gee, A. Renard, A. Randolph, J. A. Levy, D. Dina, and P. A. Luciw, *Science* 227:484-492, 1985). The primary amino acid sequence for the major internal protein predicted from the nucleotide sequence analyses of these three independent virus isolates shows 100% correlation with the amino acid sequence of HTLV-III p24 derived from purified protein in this report (Fig. 4).

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